A SURVEILLANCE AND CONTROL PROCEDURE FOR *Bovine leucosis* AND OTHER DISEASES

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OUTLINE

ABSTRACT

A surveillance and control program for the dairy cow disease *Bovine leucosis* is described. Efficiency, epidemiological, and cost minimization aspects are considered. Two statistical procedures found to be very useful are group testing and double sampling. Methods for estimating initial prevalence of the disease are discussed. The proposed program will not be prohibitively costly and can result in new information on dairy cattle as well as giving a surveillance and control procedure for the disease.

1. INTRODUCTION

Present action by European dairymen to create a *Bovine leucosis* virus (BLV)-free zone in Western Europe restricts the sale of U.S. grown dairy cows in Europe to BLV-negative animals and provides the motivation to develop a surveillance and control program for the disease *Bovine leucosis*, caused by the *Bovine leucosis* virus (BLV). Standards for exporting cows are that the cow and the herd from which the cow comes must be free of BLV for 90 days prior to the sale. A closed herd which has been BLV-free for 90 days is unlikely to contain animals which are infected and who would later come asymptomatic for the disease. New York State dairymen who are interested in exporting dairy cattle are very interested in BLV testing, in establishing BLV-free herds, and in the possibility of establish a BLV-free zone in New York State.

In order to achieve these goals and be able to export cows continuously through time, it is necessary to set up a surveillance and disease control program for all herds interested in maintaining a BLV-free herd. While the Europeans have establish a BLV-eradication program which relies on a slaughter endemity program, this approach would be prohibitively expensive in the U.S. Therefore, we propose a program of disease surveillance and improved management techniques with selective culling of BLV-positive animals. The application of statistical group testing techniques and epidemiologic techniques will make this program both cost effective and feasible. Such a program is described in the following sections. The prevalence of BLV varies tremendously between herds with an average prevalence of 20-25% for a herd. The prevalence of BLV in a herd will determine the sampling and management procedure. Several procedures

will be presented. Discussion is centered on herds with greater than 20% BLV (high prevalence), between 5% and 20% (medium prevalence), and below 5% (low prevalence). This classification of percentages is disease and investigator dependent. Various sampling procedures are useful in considerably decreasing laboratory costs. Two of these are group testing (see, e.g., Bush *et al.*, 1984; Dorfman, 1943; Hwang *et al.*, 1981; Pfeiffer and Enis, 1978; Raghavarao and Federer, 1973; Sobel, 1967; Sobel and Groll, 1959 and 1966) and double sampling (Neyman, 1938). Three forms of group testing are described. Several methods for obtaining preliminary estimates of prevalence are given. One method could lead to interesting new biological knowledge. Criteria for pooling samples are discussed and critiqued. Various epidemiological aspects require consideration before a surveillance and control program should be instituted. These are briefly discussed.

In light of the above a surveillance and control program for attaining a BLV-free status for a herd is described. Six aspects of surveillance and control programs are discussed:

1. the determination of prevalence and incidence of BLV in herds.
2. management and control programs for the prevalence level and conditions on a given dairy farm.
3. the baseline epidemiologic survey for the initial determination of BLV prevalence.
4. Application of group testing methods and procedures for processing samples in a diagnostic laboratory.
5. Determination of optimal sampling interval in the surveillance program.
6. Cost minimization of the program.

The initial disease prevalence and subsequent incidence and the time in

which a disease-free status is desired are important factors in determining sampling intervals and appropriate management techniques. Some variations of the control programs are described. Dissemination of results is an important factor in the success of the control program. Quality control procedures need to be instituted in all phases of the program in order to maintain high standards and quality of results.

2. SAMPLING PROCEDURES

Once N samples are at the laboratory for diagnosis, various sampling procedures can be utilized to cut costs and/or the number of analyses. Two such procedures, group testing and double sampling, are discussed below. Three particular group testing procedures are described. There are many forms of group testing and double sampling but we describe only selected ones.

Group testing procedure I: Dorfman (1943) describes the following procedures for N samples where it is desired to determine which samples are positive or negative, diseased or not diseased, or any other binary classification. Let g be the number of samples pooled into one group. There will be $G_1 = N/g$ such pools or groups. Let p = the proportion diseased and q = 1-p = the proportion not diseased.

If the pools have individuals randomly assigned, then the expansion of $(p+q)^g$ gives the proportion (frequency of occurrence) for $0,1,\cdots,g$ diseased animals in a pool. $1-q^g$ gives the expected proportion of disease-free animals in a group of g. Hence, $G_2 = G_1(1-q^g)$ is the number of pools expected to be positive (contains a sample from a diseased animal). On these positive samples, analyses are conducted on each of the g samples in a pool. Therefore, the expected total number of analyses will be

$$G_1 + gG_2 = \frac{N}{g} + N(1-q^g) = N\left(\frac{1}{g} + 1 - q^g\right) \quad . \tag{2.1}$$

When $1/g = q^g$ the number of analyses will be identical to N, i.e., the number required to analyze each sample separately. When $1/g = q^g$, this will be called the *break-even* group size. Also, the maximal value of p for

which groups testing can be used is determined as follows. The smallest group size greater than one is two. Therefore, $1/g = 1/2$ must be greater t than $q^g = q^2$. That is, $1/2 > q^2_{max} = .706^2$. This means that $p < 1 - .706 = .294$ results in savings from pooling. Then, $p_{max} = 0.294$ is maximum value for which group testing can be used in order to effect savings.

The *optimal group size* given p is obtained as a solution for g to the equation

$$g^2_{opt} = (q^g \log 1/q)^{-1} . \qquad (2.2)$$

To illustrate the above, consider an example for N samples where p=.05 and q=.95. From equation (2.1) we obtain the following:

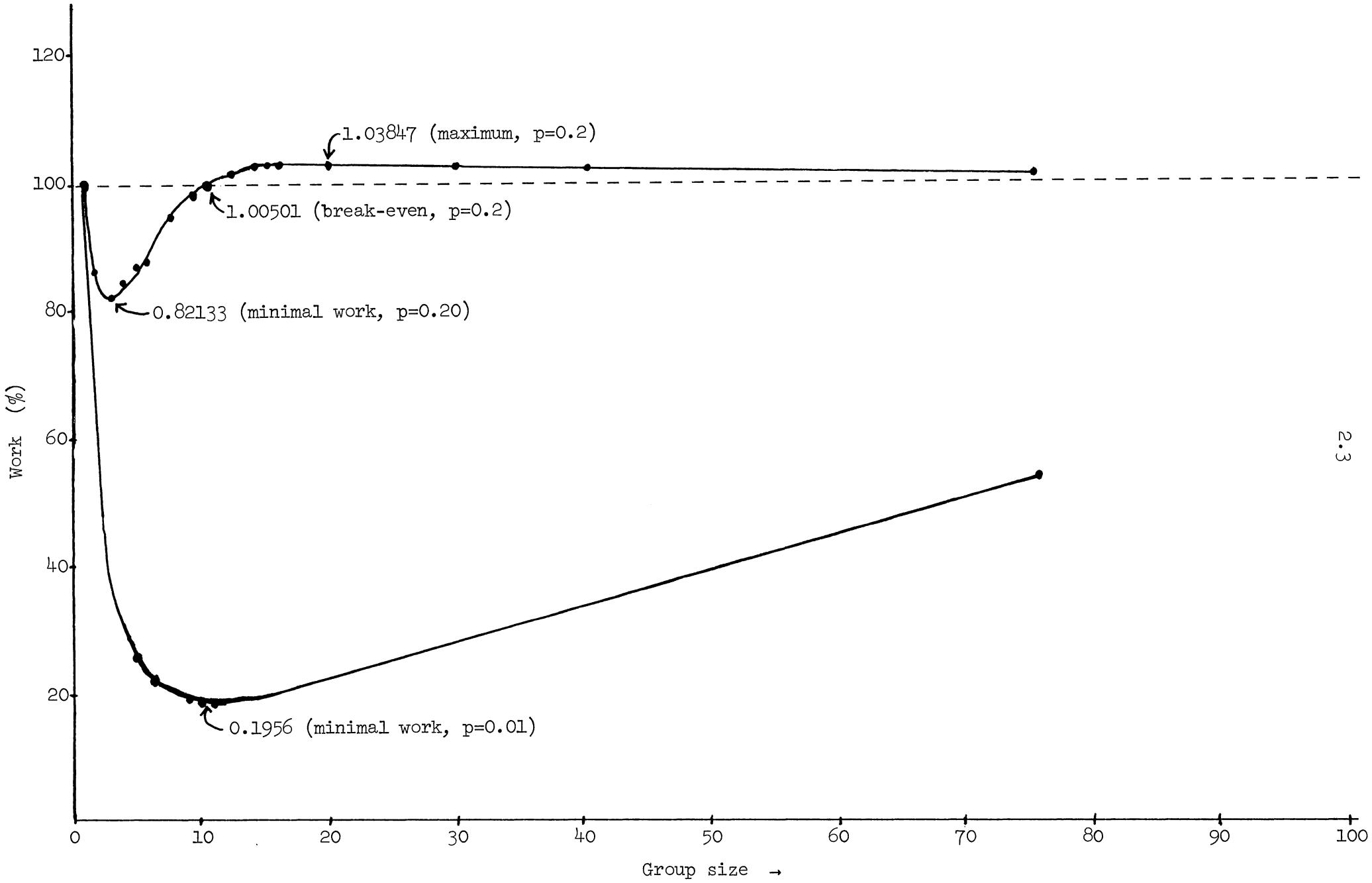
Group size	Number of analyses G_1+gG_2
1	N
2	$N(1/2+1-.95^2) = 0.5975N$
3	$N(1/3+1-.95^3) = 0.4760N$
4	$N(1/4+1-.95^4) = 0.4354N$
5	$N(1/5+1-.95^5) = 0.4262N$
6	$N(1/6+1-.95^6) = 0.4315N$
7	$N(1/7+1-.95^7) = 0.4445N$
8	$N(1/8+1-.95^8) = 0.4616N$

The optimal group size is $g_{opt}=5$ and using this value results in a savings of $1-.4262 = 57\%$ over running individual analyses. Any of the group sizes of 3 to 8 result in over 52% savings. Figure 1 indicates the various values for p=.01 and .2. The savings (or loss) in efficiency for various g from 1 to 70 is given on the graph.

For various p, g_{opt} and the percent savings achieved are from (2.1) and (2.2):

p	q	g_{opt}	% savings
.01	.99	11	80
.05	.95	5	57
.10	.90	4	40
.20	.80	3	18
.25	.75	2	9
.29	.71	2	0.4

Figure 1. Amount of work for various group sizes



Group testing procedure II: This procedure involves continuous splitting of positive pools into two pools and testing the pools. It has been studied by a number of authors (e.g., Sobel, 1967, Sobel & Groll, 1959 and 1966). For N samples the steps are

Step 1 Test a pool from all N samples. If negative stop and if positive go to step 2.

Step 2 Obtain two pools of size N/2 and test the two pools.

Step 3 Obtain two pools of size N/4 from each of the positive pools in step 2 and test.

Step 4 Obtain two pools of size N/8 from each of the positive pools in step 3 and test.

Step 5 Obtain two pools of size N/16 from each of the positive pools in step 4 and test.

Proceed until the last split of positive pools results in analyses on individual samples.

As an illustration of the above let N=72 samples:

Step 1 A pool from all 72 samples results in a positive result.

Step 2 Sample numbers 1-36 were put in one pool and 37-72 in a second pool. Suppose that both pools yields a positive result.

Step 3 Pools from samples 1-18, 19-36, 37-54, and 55-72 were obtained and pools 1-18 and 55-72 were positive.

Step 4 The two positive pools were split into four pools of 9 samples each, i.e., 1-9, 10-18, 55-63, 64-72; suppose all were positive except 55-63.

Step 5 The three positive pools were split with six pools of size four or five, i.e, 1-4, 5-9, 10-13, 14-18, 64-67, and 68-72. Suppose that pools 1-4, 5-9, 10-13, 64-67, and 68-72 were positive.

Step 6 The five positive pools were split into pools of two or three, i.e., 1-2, 3-4, 5-6, 7-9, 10-11, 12-13, 64-65, 66-67, 68-69, and 70-72. Suppose that pools 1-2, 7-9, 10-11, 64-65, and 70-72 were positive.

Step 7 Perform individual sample analyses on the positive pools in step 6.

The total number of analyses performed was 1 + 2 + 4 + 4 + 6 + 10 + (2+3+2+2+3) = 39. This resulted in a savings of 72 - 39 = 33 samples over doing the 72 samples individually.

Group testing procedure III: Raghavarao and Federer (1973) and Bush *et al.* (1984) propose other methods of group testing. One such method is the following:

Step 1 Test pool from N samples. If positive, proceed. If not, stop and state all N samples are negative.

Step 2 Arrange N samples in as square an array as possible with r rows and c columns. Note that r+c is a minimum when r=c. Test the row pools and the column pools. In some cases the positive row and column pools uniquely determine the positive samples. When the positive or negative quality of a sample cannot be uniquely determined, proceed to Step 3.

Step 3 For those rows and columns which are positive and for which the positive or negative value of a sample cannot be uniquely determined, these intersection samples are analyzed individually. Since p in these intersection samples may be fairly high, it may not be efficient to use group testing with groups of g=2, say.

The above steps are illustrated with an example. Let N=72, r=8, and c=9. Suppose that samples 1, 2, 9, 11, 65, and 72 are positive but this is unknown. Since we know nothing about the samples we may as well array them as follows:

	Columns									
Rows	1	2	3	4	5	6	7	8	9	
1	1	9	17	25	33	41	49	57	65	+
2	2	10	18	26	34	42	50	58	66	+
3	3	11	19	27	35	43	51	59	67	+
4	4	12	20	28	36	44	52	60	68	
5	5	13	21	29	37	45	53	61	69	
6	6	14	22	30	38	46	54	62	70	
7	7	15	23	31	39	47	55	63	71	
8	8	16	24	32	40	48	56	64	72	+
	+	+	+						+	

The row and column pools showing positive results are indicated above. We need to test samples 1, 2, 3, 8, 9, 10, 11, 16, 65, 66, 67, and 72. Thus with 8 + 9 = 17 analyses, the problem was reduced from 72 samples to 12 samples. The total number of samples analyzed was 1 + 8 + 9 + 12 = 30 instead of 72 to find the six positive samples 1, 2, 9, 11, 65, and 72.

Obviously, as the number of positives increases the greater will be the number of analyses. The maximum number of analyses is 1 + r + c + N and the minimum number (all positives in the same row or column) is

1 + r + c. For example, suppose that there were eight positives. If these all fell in the same column, only 1 + 8 + 9 = 18 analyses would be required. If these eight fell in different rows and different columns, then 1 + 8 + 9 + 64 analyses would be required.

For each number of positives out of N all possible configurations need to be studied, their probabilities ascertained, and the relative efficiencies determined. For certain N, one could start with rc groups of size g and follow through as for single samples. Then, the positive or possibly positive groups would each be arrayed in a row-column array for individual samples and steps 2 and 3 will be repeated. The effect of r and c values for various numbers of defectives needs to be studied.

Double sampling: Double sampling procedures, like group testing procedure I, have been in the literature and in use for many years. In the thirties, U.S.D.A. statisticians A. J. King and C. F. Sarle were using and discussing the following procedure. Variate X_1 is cheap to obtain but is not what is desired. Variate X_2 is expensive and desired. X_1 and X_2 are related. A large sample of X_1 is obtained and a relatively small sample of both X_1 and X_2 is obtained. An illustration is for X_1 to be a response to a question from a mailed interview and X_2 to be the response from a personal interview. The large sample for X_1 is used to reduce the variance in X_2 using regression analyses. The procedure was described in a publication by Neyman (1938). The above is one of many forms that double sampling can take.

Double sampling involves the use of two or more procedures, assays, methods, etc., when the cost, efficiency, availability, and/or accuracy of the procedures differ. In disease studies, two methods of ascertaining the

presence or absence of a disease might be available. One could be a quick-and-dirty (not accurate) one and the second a complicated, precise, and expensive method. The latter might be used on pooled samples whereas the former might suffice on the individual samples. Or, one could use the procedure described for the original concept of double sampling in surveys. In disease studies, if the prevalence is high and a second sampling is anticipated, then the quick-and-dirty procedure might be used first. If the prevalence was low, the expensive-precise method might be used.

Other examples could involve responses from milk samples X_1 and responses from blood samples X_2. Milk samples are obtained more cheaply and easily than blood samples, especially if a veterinarian is required to obtain the blood samples. The number of skilled, reliable, and accurate technicians may be limited relative to the number of samples requiring processing. The use of unskilled technicians to obtain analyses, say X_1, and of skilled technicians to obtain analyses, say X_2, would represent another example. Blood samples might be obtained by unskilled individuals to obtain response X_1 on a large sample and from a small sample by both the unskilled person and a trained veterinarian.

3. PRELIMINARY ESTIMATES OF PREVALENCE

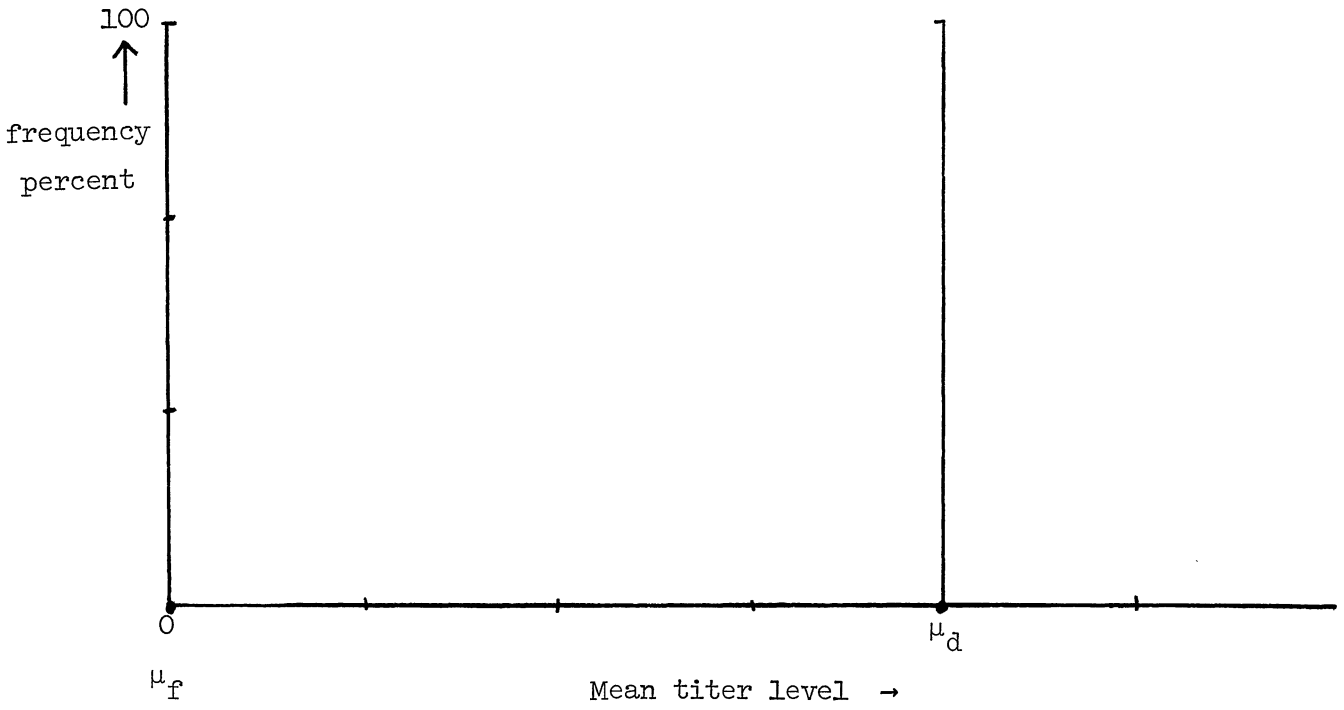
There are many procedures for obtaining a preliminary estimate of the prevalence of a disease in a herd or population of herds. Previous data are often a source for prevalence estimates. These data could come from previous studies, investigations, or surveys. In addition, some farmers often have an idea of the prevalence in their herds. A local veterinarian may be able to provide an estimate of prevalence in a region. State veterinarians may have data on prevalence in various regions of the state.

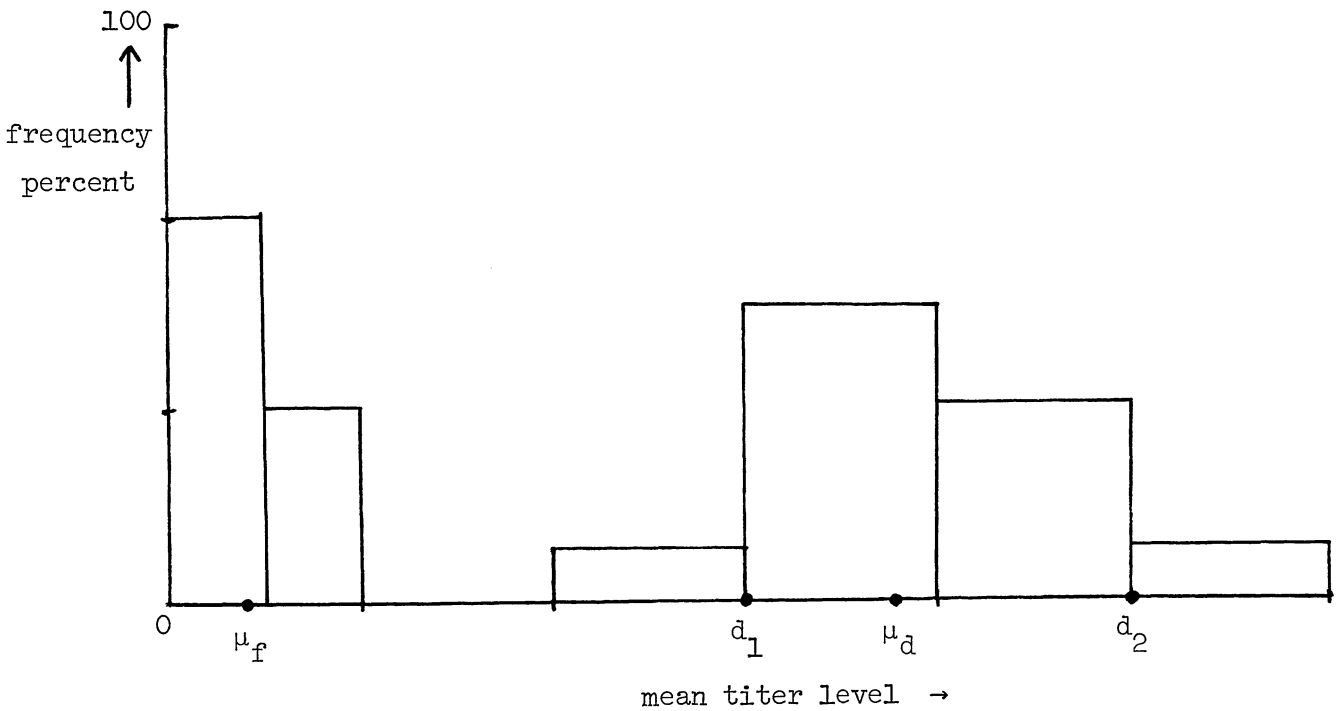
In lieu of previous estimates of prevalence, an investigator may draw a small subsample of the samples obtained and estimate prevalence for the region or for a herd. This preliminary estimate could be used to determine group size for a group testing procedure or for determining whether or not to utilize a double sampling procedure. In a region this subsample may be a random sample of a fraction of a percent, say, whereas in a herd it could be ten samples.

In certain instances, another procedure can be used to obtain a prevalence estimate in a given herd. To use this procedure, certain information must be available. The minimum information necessary is the number of cows in a herd N, the mean titer level for nondiseased cows μ_f, and the mean titer level for diseased cows μ_d. This is depicted in the top graph in Figure 2. In a pooled sample (equal amount from each of the N cows) the mean titer level would be determined and is equal to M, say. Now there are N_d diseased cows and $N_f = N-N_d$ disease-free cows in the herd. N_d is unknown, and a solution needs to be found. Since

$$N_f\mu_f + N_d\mu_d = (N-N_d)\mu_f + N_d\mu_d = M \quad , \tag{3.1}$$

Figure 2. Frequency distribution of mean titer level for disease-free and diseased cows.





$$N_d = (X-N\mu_f)/(\mu_d-\mu_f) \quad . \tag{3.2}$$

A solution for prevalence is $N_d/N = p$.

The above is the ideal situation which may never be achieved in practice. Instead, the situation in the bottom graph of Figure 2 may be more realistic because of variations in titer levels among cows and/or variation in estimating titer level in a given sample. Equation (3.2) can still be used to estimate N_d, say $\hat{N}_d$, and the estimated prevalence would be $\hat{p} = \hat{N}_d/N$. A confidence limit for $\hat{p}$ can be obtained using first d_1 and then d_2 in place of μ_d in (3.2). Since the interval $[d_1,d_2]$ contains 5/6ths, say, of the distribution, this would be a 5/6ths = 83% confidence interval on p.

It would be desirable to collect information on titer level among disease-free and among diseased cows for other diseases. This requires that the actual titer level be recorded and not just whether a cow is classified as diseased or not diseased. Given empirical frequency distributions of titer levels, it may be possible to determine a mathematical form for this distribution. From the analytic form of the distribution, confidence intervals of any width may be determined for N_d, and consequently for p. It would appear that biological theory for disease would be considerably enhanced through this information. For a known population mean titer level of diseased and disease-free cows, a prevalence estimate from the herd pooled sample can be obtained. Then, an optimal group size for group testing can be selected.

4. CRITERIA FOR POOLING SAMPLES

Two main conditions must be present before samples can be pooled. The first one is the sensitivity of a test procedure. Sensitivity needs to be defined. In performing an assay, information is needed on the proportion of times the assay fails to detect the response when it is present. One would hope that this is zero but it must be ascertained and not assumed. Then sensitivity must be defined in terms of this actual proportion.

Information is needed on various dilution rates of a sample. If the assay loses its sensitivity for dilutions less than 1/g, say, the group or pool size must be less than g. For BLV tests the work of Mammerickx *et al.* (1984) is useful. They conclude that with the sensitivity for their ELISA (enzyme-linked immunosorbent assay), the group size g should be less than or equal to 75. Before any pooling procedure can be used with a given assay, it is necessary to determine the smallest dilution proportion for which the procedure remains sensitive.

The second major condition that must be realized is that no antagonisms are set up when samples are pooled. If antibodies and/or antigens from different samples set up a reaction which lowers or raises the titer of a pooled sample, the results would probably not be usable. This would indicate that pooling procedures would not be useful for this particular study. Again a knowledge of antibody, antigen, and titer level interactions would enhance the biological theory of disease.

5. EPIDEMIOLOGICAL ASPECTS

In any disease program it is necessary to consider epidemiological aspects of the disease in the population. Some points for consideration are

i) How should incidence and prevalence of a disease be measured?

ii) How should measurements from a sample be made?

iii) What specificities or peculiarities are associated with this disease?

iv) Is the disease bacterial or viral?

v) Is it necessary to use blood samples or can other samples (e.g., urine or milk) be used as effectively?

vi) How is the disease transmitted?

vii) How infectious is the disease?

In measuring incidence and prevalence it is necessary to determine whether or not the herd or the individual animal should be the unit of consideration. The proportion of herds with one or more diseased animals may be the prevalence desired. For other situations, the proportion of diseased animals in a herd may be the desired statistic.

6. A PROPOSED SURVEILLANCE AND CONTROL PROGRAM

The goal of any surveillance and control program for a disease such as BLV or other similar diseases should encompass the following:

i) Determination of prevalence of disease in the population being studied – The population of dairy cows could be all the dairy cows in New York State or initially it could be cows in those herds where the owner has decided to cooperate in the program. For BLV, this could be the herds desiring to export dairy cows. This population must first be delineated and a sampling frame (list of herds) prepared. To determine prevalence in the population, a simple random sample or perhaps a stratified sampling plan such as proposed by Robson (1959) would suffice.

ii) Determination of incidence of disease in the prescribed population – Incidence is the rate of new infections occurring in the prescribed period of time. To obtain an estimate of incidence, it would be necessary to use a two-stage procedure such as the one described by Robson (1959). The rate of new infections would be desirable knowledge in setting up the control program. This would measure the effectiveness of the management program. The time interval between samplings would be related to the incidence rate.

iii) Prevalence in a given herd – Since a control and surveillance program would need to be herd by herd, the prevalence of the disease in each herd in the target population needs to be ascertained to institute a control program.

iv) Control programs – A number of control programs could be instituted. For example, one control program, say A, could be to use a different or sterilized needle for each cow vaccinated or blood sample taken. Any other procedure that would infect a cow would be discontinued. A second control program, say B, would use A plus segregation of animals into two groups, i.e., disease and nondiseased. A separation of the two groups by as much as 60 feet would be sufficient for BLV. The diseased group would be milked last and contact with nondiseased animals eliminated. Any culling from the herd would be mostly from the diseased group. A third control program, say C, could be more drastic in that all animals found to be diseased would be eliminated immediately from the herd; control program A would also be in use. Obviously, many other procedures could be devised for BLV.

Any control procedure selected would more than likely depend upon the prevalence p in a herd. For high values of p, say above 20%, control procedure A would probably be the only economically feasible program. It could be that program B would be used for these values of p but this would depend upon the facilities available on a given dairy farm. In some cases, division of the herd into two separate groups may be relatively inexpensive, whereas in other cases it could be expensive. For medium values of p, say from 5-20%, method B might be feasible. If p is less than 5%, the owner could opt for program C. In any event a number of management control programs would need to be described and presented to the owner, who would implement one or more of them.

v) Laboratory analyses – Routinely blood samples are taken from cows and sent to the New York State Diagnostic Laboratory for analysis to determine whether or not a cow has BLV. In order to make the processing of laboratory analyses feasible, and not inordinately expensive, it is suggested that one of the group testing procedures described in Section 3 be instituted. If it is fairly certain that one or more cows in a herd are diseased, step 1 should be skipped. In the first stages of the program, p could be relatively large, whereas p becomes much smaller as diseased BLV cows are culled from the herd. The efficiency of group testing increases as p decreases. Considerable savings can be effected when p becomes small, say one percent (see Figure 1) or less. The number of analyses required is reduced by 80% or more. Thus, the laboratory facilities may not need to be expanded to perform the desired analyses in a BLV surveillance program.

vi) Surveillance intervals – The sampling intervals after instituting control programs A, B, C, or some other one is dependent upon the incidence of BLV, the prevalence p in a herd, and the need for action. Since the incubation period is relatively long, say three to six months, for BLV, it is recommended that the first time interval for disease control program A be at one-year intervals and for B and C be three months. Here the reasoning is that the elimination of diseased animals will require a much longer time period for A than for B and C. Since p should be considerably reduced or even zero for the disease-free group of the previous period, the second time interval

could be six months. The length of time between samplings should be doubled whenever a herd has been disease-free for the preceding two samplings. However, the interval should not be more than two or three years between samplings in a given herd.

Since export standards require a herd to be BLV-free for 90 days and for the animal to be BLV-free at the time of export, the above intervals may have to be changed to 90 days to conform to standards. Thus, when cows are being readied for sale, a sampling could be made and then another one 90 days later. This plus previous history on the BLV status of the herd should more than meet export standards. Any cow coming into a herd should meet the export standards also in order to avoid re-introducing BLV cows into a herd. The tests should be made on all young animals carried along with or introduced into the herd.

7. VARIATIONS OF CONTROL PROGRAMS

In utilizing any control program such as A, B, or C of the previous section, it is necessary to ascertain the effectiveness of the program in reducing prevalence. A herd management program such as A may take several years to obtain a BLV-free herd. However, if infection is mainly transmitted from cow to cow with a needle or from nursing a BLV dam, the culling of BLV animals and the use of good clean management procedures may dramatically reduce incidence while the prevalence of BLV would be reduce through normal culling of cows. This would be a relatively cost effective method to attain BLV-free status for a herd.

The separation of animals into BLV-free (at last test) and BLV-positive groups could be expensive if the groups were of approximately the same size. If the BLV group was composed of a few animals, it may not be too difficult and expensive to maintain the two groups separately. As the culling of BLV cows continues, the second group becomes smaller and smaller. When p is low, program C may be the best program to follow.

A disease such as BLV can be controlled by good management practices. Whenever other diseases can be likewise controlled, it may be advantageous to place any diseased animal in the second group and to cull them as expeditiously as possible. The BLV group may have cows with a variety of diseases. For some diseases, the isolation may have to be much more distant than that for BLV, which has been stated to be 60 feet or more. It may be that the diseased cows from several herds would be put into one designated and isolated herd until culled.

8. MINIMIZATION OF COSTS OF A CONTROL PROGRAM

The costs in any control and surveillance program can become prohibitive or very expensive at the least. It is necessary to study all aspects of the program and to minimize cost at *every* stage. The first costs to be considered are those to the farmer. A control and management program should be selected to meet the conditions on a particular farm. For example, if needles become too expensive for single use, some easy, inexpensive method of sterilizing needles must be found. Methods of isolation must be devised to minimize expenses while meeting the requirements for isolation. A number of such options should be available for each farmer.

A second cost is the procurement of individual samples. For BLV, blood samples are considered necessary. It would be desirable to ascertain if milk samples could be used in place of blood samples. Also, a skilled technician for taking blood samples should be considerably less expensive than a veterinarian. It may not be advisable for a farmer to be the one to take blood samples after the first or second samplings. He would be suitable to take blood or milk samples if he was unable to determine whether or not a cow has BLV. If effects of the disease are not apparent to him, it is doubtful that he could bias the sample. Whoever takes the samples, precautions should be taken to prevent biases or possible biases in the samples submitted to the laboratory. Costs associated with storing and transmitting to the laboratory should also be minimized but not to the point of damaging the samples.

Costs for reliable methods in the laboratory need to be minimized. The ELISA test (see Mammerickx *et al.* (1984)) is a reliable test even for

dilutions up to one in 75. In addition to being sensitive, the test is relatively inexpensive (one dollar per sample). With such a test, group testing is feasible and can result in a considerable reduction, say 80-90%, of the number of samples processed. In computing savings, the cost associated with pooling samples needs to be ascertained and taken into account when considering the savings due to group testing. If these costs are appreciable, less inexpensive methods of pooling need to be studied. When such a test as the above ELISA is expensive compared to a second less sensitive and expensive test, the first sampling may utilize the second test on individual samples or on small pools, say less than five. Any animals missed in the first sampling will be picked up in later samplings. As the herd approaches the disease-free status, the more precise and expensive test would be put into use.

Possibly the greatest reduction in costs can be achieved by lengthening the time interval between samplings. A procedure of sampling every month is three times more costly than sampling every three months. Sampling every six months is one-half as expensive as sampling at three-month intervals. The longest interval that will achieve the desired goal should be used, but standards should not be lowered simply to minimize cost. The cheapest thing is to do nothing but this is ruled out.

9. DISSEMINATION OF INTERIM RESULTS

In any cooperative program, it is necessary to inform the participants of the results as the surveillance program proceeds. Primarily farmers will be specifically interested in the progress they are making toward the disease-free status. This feedback should occur immediately after the samples are analyzed. At the same time, it should be pointed out how other control procedures would speed up or delay the approach to a BLV-free status. Secondarily, a farmer would be interested in the progress of other participants (as a group) toward BLV-free status. The information feedback is necessary to attain continued enthusiasm for the program. Also, any information on economic losses due to BLV should be disseminated. At present, it is thought that milk production is not affected by the BLV disease. This may not be a true statement but instead the effect may be small. For example, the disease may reduce production by two pounds per day, which is 730 pounds per year. If the cow is diseased for three years, this would be a loss of 2,190 pounds for the cow. Small differences in daily production can result in fairly sizable losses over the lifetime of a cow in the herd. Small losses would not be apparent to a dairyman. As information becomes available, it should be made known to dairy farmers. This could entice more farmers to join the program for milk production rather than for exporting reasons.

The general dairy farm and animal production population should be kept informed of the progress of lowering prevalence of BLV in dairy herds in the study.

10. QUALITY CONTROL AND CHECKS

In any sample analysis program, there should be constant monitoring of the various sample analyses being made and on the persons performing the analysis. Check samples of known composition should be randomly interspersed in with the other samples. The identity of the check samples should be unknown to the analyst. It is suggested that check samples with one BLV sample and g-1 BLV-free samples and with two BLV samples and g-2 BLV-free samples be included with a frequency of one to five percent for the population of herds; g is the group size used for the pools. Whenever a check sample is off by a designated amount, the entire lot would be rerun by another analyst to check on the method and the analyst. One rough rule for number of checks is to use $\sqrt{N}+1$ checks when N samples are analyzed. This number could become rather large for very large N. Here it might be desired to use only one-tenth or one-half percent frequency for random checks. In performing a herd analysis, it may be desirable to include one or two checks for every sampling of a herd. In a group testing procedure, the check could be a single sample of a BLV cow with known titer or it could be a pool of g samples as described above. Perhaps various checks would be used for different herds and different samplings. A quality control procedure for group testing has not been developed to date.

Charts on prevalence for each herd need to be prepared and updated as the surveillance continues. These would give the history of prevalence in a herd and the progress being made toward the goal of maintaining a BLV-free herd. Check sample results need to be included wherever they occur to validate the results. Perhaps copies of these graphs or charts should be sent to the owner of a herd as part of the information feedback discussed in Section 9.

11. PROGRAM EVALUATION

The success of any proposed program depends on the cooperation of New York State dairymen. To obtain cooperation it may be necessary to assure confidentiality of lab tests for each herd. This can be done despite the state law open all New York State Diagnostic Laboratory record to the public. The results of laboratory analyses for each herd must be disseminated to the owner promptly.

The proposed control program and laboratory procedures should be field tested prior to using them at the state level.

12. CONCLUSIONS AND SUMMARY

It is concluded that the goal of a BLV-free herd can be achieved through good management practices. The cost of a surveillance and control program can be kept reasonable by minimizing costs at every stage. Group testing will be an effective procedure to reduce sample analysis costs and in preventing a backlog of samples to be analyzed. Timely information and feedback of information can be accomplished without being unduly costly. Group testing can be effectively used in a BLV surveillance and control program. Milk production records can be kept on BLV and BLV-free cows to assess the impact of the disease on production. Other related information can also be collected, processed, and given to dairymen.

In the foregoing, a surveillance and control program has been outlined. Implementation of the plan in New York State will indicate aspects of the program requiring change. Various group testing and double sampling procedures have been described. Several methods for obtaining preliminary estimates of prevalence have been discussed. It is shown how and where costs of a surveillance and control program can be reduced. Constant checking is required to maintain the quality of the program. Some comments on this aspect are given but an unsolved statistical problem is to develop optimal quality control programs for group testing. Quality control checks will need to be instituted in every phase of the program.

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